IIA 5.8.3 Oral

Report:	IIA 5.8.3/02 Smedley, J. (2007) An Acute Oral Toxicity Study in Rats with MON 52708. Charles River Laboratories, Spencerville, Ohio, US. Study Number: EUF00137. Issue date 11 January 2007. Unpublished. (Monsanto Study No.: CRO-2006-050). MRID #47899504.

Guidelines: Acute Oral Toxicity (rat) OECD 425 (2001): OPPTS 870.1100 (2002)

Sponsor: Monsanto Company

Executive Summary: In an acute oral toxicity study (Up and Down Procedure), young adult female Sprague Dawley rats were given a single oral dose of MON 52708, a metabolite of Dicamba, at 550 mg/kg bw, 2000 mg/kg bw or 5000 mg/kg bw. The test substance was administered as a 25% w/w mixture in distilled water. Animals were observed for mortality, signs of gross toxicity, and behavioural changes at least once daily for 14 days or until death. All animals were necropsied.

The single animal dosed at 550 mg/kg bw survived, gained weight, and exhibited no clinical signs of toxicity during the study period. No gross lesions were observed at necropsy

Two of the five animals dosed at 2000 mg/kg bw died within one day post-dosing. Clinical observations noted prior to death included wobbly gait, rapid breathing, urine/fecal stain, apparent hypothermia, ocular discharge, dark material around the nose, intermittent tremors, soft stools and salivation. In surviving animals, clinical observations were limited to transient incidences of urine stain, dark material around the nose and excessive food pile under the cage in one animal. Survivors gained weight throughout the study period. At necropsy, abnormal content of the digestive tract was observed in decedents. One incidence of a cyst on the uterus was noted in one animal that survived to study termination; this finding was not considered to be related to the test substance due to the isolated nature of the finding.

Three of the four animals dosed at 5000 mg/kg bw died post-dosing on day 0. Clinical observations noted prior to death included tremors, prostration, laboured breathing, fecal stain, apparent hypothermia, ocular/nasal discharge, wobbly gait, decreased activity, soft stools and salivation. In the single surviving animal, clinical observations were limited to one incidence of dark material around the nose. The survivor gained weight throughout the study period. At necropsy, abnormal content of the digestive tract, stomach discolouration, slight autolysis of most organs of the abdominal cavity, and foci on the thymus were observed in decedents. No gross lesions were observed in the lone survivor.

Oral LD_{50} Females = 2641 mg/kg bw (based on maximum likelihood)

Based on an estimated LD_{50} of 2641 mg/kg bw in female rats, MON 52708 meets the criteria for USEPA Toxicity Category III.

This acute oral study is classified as Acceptable. This study satisfies the guideline requirement for an acute oral study (OPPTS 870.1100; OECD 425) in the rat.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. Materials:

Test Material:	MON 52708
Description:	White powder
Lot No./:	GLP-0603-16958-T
Purity:	97.9%
CAS#:	Not reported
Vehicle:	Distilled water
Stability of test compound:	Expiration date: 20 March 2007

Test Animals:	
Species	Rat
Strain	Sprague Dawley
Age at dosing	8-10 weeks
Weight at dosing	180-213 g females
Source	Harlan Sprague Dawley, Inc., Indianapolis, Indiana, US
Housing	Singly housed in suspended stainless steel cages.
Acclimatization period	at least 5 days
Diet	PMI Certified Rodent Chow #5002
Water	Municipal tap water treated by reverse osmosis, *ad libitum*
Environmental conditions	Temperature: 18-23°C Humidity: 36-71 % Air changes: 10-15 per hour Photoperiod: 12 hour light/12 hour dark

B. Study Design and Methods:

1. In-life dates: Start: 4 May 2006 End: 16 June 2006

2. Animal assignment and treatment: Prior to each dosing, experimentally naïve rats were fasted and weighed. Ten healthy naïve rats were selected for test. Animals were assigned to the test groups as noted in Tables IIA 5.8.1/02-1, below. The test substance was administered as a 25% w/w mixture in distilled water via gavage. Initially, the test substance was administered to a single female at a dose of 2000 mg/kg bw. Following the Up and Down procedure, nine additional animals were dosed at either 550 mg/kg bw, 2000 mg/kg bw or 5000 mg/kg bw. The test substance was administered in sequence. Individual body weighs were recorded prior fasting (day -1), prior to dosing on (day 0) and again on Days 7 and 14or after death. Animals were observed for clinical abnormalities a minimum of two times on day 0 post-dosing, with the first observation

within 30 minutes post-dosing, and daily thereafter for the 14 day study period. All animals were necropsied.

Table IIA 5.8.3/02-1. Main Test: Doses, mortality/animals treated

Dosing Sequence	Animal No.	Sex	Dose (mg/kg bw)	24 Hour Result	48 Hour Result
1	A5923	F	2000	X	X
2	A5926	F	550	O	O
3	A5995	F	2000	O	O
4	A5986	F	5000	X	X
5	A6075	F	2000	O	O
6	A6045	F	5000	O	O
7	A6078	F	5000	X	X
8	A6094	F	2000	O	O
9	A6087	F	5000	X	X
10	A6166	F	2000	X	X

(O = survived, X = dead)

3. Statistics: After each animal was dosed, the short-term and long-term outcomes (mortality) were input into the OECD 425 Acute Oral Toxicity Statistical Program (OECD 425 AOT Program). When the stopping criteria were engaged, the program calculated the LD50 and 95% confidence intervals.
Body weight means and standard deviations were calculated.

II. RESULTS AND DISCUSSION

A. Mortality: The single animal dosed at 550 mg/kg bw survived. Two of the five animals dosed at 2000 mg/kg bw died within one day post-dosing. Three of the four animals dosed at 5000 mg/kg bw died post-dosing on day 0.

B. Clinical observations: The single animal dosed at 550 mg/kg bw exhibited no clinical signs of toxicity during the study period.
Clinical observations noted prior to death in animals dosed at 2000 mg/kg bw included wobbly gait, rapid breathing, urine/fecal stain, apparent hypothermia, ocular discharge, dark material around the nose, intermittent tremors, soft stools and salivation. In surviving animals, clinical observations were limited to transient incidences of urine stain, dark material around the nose and excessive food pile under the cage in one animal.
Clinical observations noted prior to death in animals dosed at 5000 mg/kg bw included tremors, prostration, laboured breathing, fecal stain, apparent hypothermia, ocular/nasal discharge, wobbly gait, decreased activity, soft stools and salivation. In the single surviving animal, clinical observations were limited to one incidence of dark material around the nose.

C. Body weight: Survivors gained weight throughout the study period.

D. Necropsy: No gross lesions were observed at necropsy in the single animal dosed at 550 mg/kg bw.

At necropsy, abnormal content of the digestive tract was observed in decedents dosed at 2000 mg/kg bw. One incidence of a cyst on the uterus was noted in one animal that

survived to study termination; this finding was not considered to be related to the test substance due to the isolated nature of the finding.

At necropsy, abnormal content of the digestive tract, stomach discolouration, slight autolysis of most organs of the abdominal cavity, and foci on the thymus were observed in decedents dosed at 5000 mg/kg bw. No gross lesions were observed in the lone survivor.

E. Investigator's Conclusions (extracted from page 16 in the study report): "*Under the conditions of this test, the acute oral LD50 of MON 52708 was estimated to be 2641 mg/kg (based on maximum likelihood) in the rat.*"

F. Reviewer's Conclusions: The reviewer is in agreement with the investigators. Based on an estimated LD_{50} of 2641 mg/kg bw, MON 52708 meets the criteria for USEPA Toxicity Category III.

G. Deficiencies: No deficiencies were identified.